

Renumbered Claims

10/29/2003 15:43 2128400221

NOOZYMES NA

PAGE 04/11

AMENDMENTS TO THE CLAIMS:

Claims 67 and 77 are amended. The following is the status of the claims of the above-captioned application, as amended.

Claims 1-66 (Canceled).

¹
Claim ~~67~~ (Currently amended). A method for producing a protein, comprising:

(a) culturing a bacterial host cell comprising at least two copies of the gene encoding the protein stably integrated into the chromosome in different positions, wherein at least one DNA construct is integrated into a non-functional conditionally essential chromosomal gene(s) of the bacterial host, wherein the DNA construct comprises:

- (i) a non-functional copy of the conditionally essential gene(s); and
- (ii) at least one copy of the gene encoding the protein located between the non-functional copy and a DNA fragment homologous to a DNA sequence located adjacent to the non-functional conditionally essential gene(s) of the chromosome;

wherein a first recombination between the non-functional conditionally essential gene and the non-functional copy results in a functional conditionally essential gene(s) located on the chromosome, and wherein the bacterial host cell had a copy of the gene of interest in the chromosome prior to integration of the at least one DNA construct; and

- (b) recovering the protein.

²
Claim ~~68~~ (Previously presented). The method of claim ¹~~67~~, wherein the host cell further comprises at least one additional DNA construct(s) integrated into at least one different non-functional conditionally essential chromosomal gene(s) of the host cell.

⁸
Claim ~~69~~ (Previously presented). The method of claim ¹~~67~~, wherein a second recombination between the DNA fragment and the DNA sequence located adjacent to the non-functional conditionally essential gene(s) occurs.

⁹
Claim ~~70~~ (Previously presented). The method of claim ~~69~~⁸, wherein the DNA construct further comprises at least one marker gene which is located in the construct so that it is removed from the chromosome by the second recombination.

¹⁰
Claim ~~71~~ (Previously presented). The method of claim ~~70~~⁹, wherein the at least one marker gene confers resistance to an antibiotic selected from the group consisting of chloramphenicol, kanamycin, ampicillin, erythromycin, spectinomycin and tetracycline.

⁴
Claim ~~72~~ (Previously presented). The method of claim ~~67~~¹, wherein the DNA construct further comprises at least one marker gene located between the non-functional copy and the DNA fragment, and wherein the at least one marker gene is located between nucleotide sequences that are recognized by a resolvase.

⁵
Claim ~~73~~ (Previously presented). The method of claim ~~72~~⁴, wherein the at least one marker gene is excised from the chromosome by the resolvase.

³
Claim ~~74~~ (Previously presented). The method of claim ~~67~~¹, wherein the non-functional conditionally essential chromosomal gene(s) of the host cell are non-functional due to a partial deletion of the gene(s), or an introduction of one or more mutations in the gene(s).

⁶
Claim ~~75~~ (Previously presented). The method of claim ~~67~~¹, wherein the host cell is a *Bacillus* host cell.

⁷
Claim ~~76~~ (Previously presented). The method of claim ~~75~~⁶, wherein the host cell is a *Bacillus licheniformis* host cell.

¹¹
Claim ~~77~~ (Currently amended). A method for producing a bacterial host cell comprising at least two copies of a gene of interest stably integrated into the chromosome at different positions, comprising:

- (a) introducing a DNA construct into the bacterial host cell, wherein the host cell comprises at least one chromosomal copy of the gene of interest and one or more non-functional conditionally essential chromosomal gene(s), and wherein the DNA construct comprises:

- (i) a non-functional copy of the conditionally essential gene(s); and
 - (ii) at least one copy of the gene of interest located between the non-functional copy and a DNA fragment homologous to a DNA sequence located adjacent to the non-functional conditionally essential gene(s) of the chromosome;
- wherein a first recombination between the non-functional conditionally essential gene and the non-functional copy results in a functional conditionally essential gene(s) located on the chromosome, whereby a bacterial host cell comprising at least two copies of a gene of interest stably integrated into the chromosome at different positions is produced.

¹²
Claim ~~78~~ (Previously presented). The method of claim ¹¹~~77~~, further comprising integrating at least one additional DNA construct(s) into at least one different non-functional conditionally essential chromosomal gene(s) of the host cell.

¹⁸
Claim ~~79~~ (Previously presented). The method of claim ¹¹~~77~~, further comprising a second recombination between the DNA fragment and the DNA sequence located adjacent to the non-functional conditionally essential gene(s).

¹⁹
Claim ~~80~~ (Previously presented). The method of claim ¹⁸~~78~~, wherein the DNA construct further comprises at least one marker gene which is located in the construct so that it is removed from the chromosome by the second recombination.

²⁰
Claim ~~81~~ (Previously presented). The method of claim ¹⁹~~80~~, wherein the at least one marker gene confers resistance to an antibiotic selected from the group consisting of chloramphenicol, kanamycin, ampicillin, erythromycin, spectinomycin and tetracycline.

¹⁴
Claim ~~82~~ (Previously presented). The method of claim ¹¹~~77~~, wherein the DNA construct further comprises at least one marker gene located between the non-functional copy and the DNA fragment, and wherein the at least one marker gene is located between nucleotide sequences that are recognized by a resolvase.

¹⁵
Claim ~~83~~ (Previously presented). The method of claim ¹⁴~~82~~, further comprising the excision of the at least one marker gene from the chromosome by the resolvase.

¹³
Claim ~~84~~ (Previously presented). The method of claim ¹¹~~77~~, wherein the non-functional conditionally essential chromosomal gene(s) of the host cell are non-functional due to a partial deletion of the gene(s), or an introduction of one or more mutations in the gene(s).

¹⁴
Claim ~~85~~ (Previously presented). The method of claim ¹¹~~77~~, wherein the host cell is a *Bacillus* host cell.

¹⁷
Claim ~~86~~ (Previously presented). The method of claim ¹⁴~~85~~, wherein the host cell is a *Bacillus licheniformis* host cell.

²¹
Claim ~~87~~ (Previously presented). A host cell produced by the method of claim ¹¹~~77~~.